Cdc42 GTPase Activating Proteins (GAPs) Regulate Generational Inheritance of Cell Polarity and Cell Shape in Fission Yeast

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

RE: Manuscript #E20-10-0666
TITLE: Cdc42 GTPase Activating Proteins (GAPs) Regulate Generational Inheritance of Cell Polarity and Cell Shape in Fission Yeast

Dear Fulvia,

Thank you for submitting the above manuscript to MBoC. It has now been reviewed by two external reviewers, whose comments are appended below. As you will see they have somewhat diverging opinions and ask for a number of revisions and clarifications.

I have also read the paper carefully. I think it describes interesting observations, but also feel that none of the three hypotheses explored in the models are conclusively refuted or supported by the experiments. I would ask that, in addition to addressing the comments made by the reviewers, you consider the two following main points and more minor points below.

First, your main conclusion is that unequal inheritance of the GAP Rga6 underlies the asymmetric growth patterns of rga4Δ daughter cells. You show that the two daughters of rga4Δ monopolar cells have different Rga6 levels and that rga6Δ enhances the shape asymmetry between daughter cells, but what happens to the growth patterns in these cells? This would be easy and important to test. If differential Rga6 levels contribute to growth pattern asymmetry, deleting it should make the two daughter cells more equal in growth patterns.

Second, some of the experiments are difficult to interpret and these difficulties should be openly acknowledged in the text. In particular, the interpretation of effect of cell size is complicated by the fact that pom1Δ cells are themselves largely monopolar. It thus seems rather unsurprising that the double mutants are also monopolar, and difficult to specifically attribute this change to a change in cell size. The difficulty in interpretation is similar for the gef1Δ rga4Δ experiments, as cells lacking gef1Δ are themselves monopolar.

Other clarification needed:

It would be nice to provide supplementary movies to Fig 2A to show the interesting pedigree growth pattern of rga4Δ cells.

In Fig 5B, the rga4Δ cell marked as bipolar does not look very bipolar compared to the monopolar one shown above: the distance from the birth scar to the cell end seems quite similar in the two cells.

I have trouble understanding the quantification of the pom1Δ rga4Δ experiment in Fig 6. The text states that all monopolar cells gave rise to two monopolar daughters (100%), but the panels B and C show some bipolar cells. What is the frequency of the bipolar pattern?

p. 10: It would be helpful to the reader if you provide a more extensive explanation of the parameters that are changed in the model.

p. 12: You explain you performed an SGA screen, by visual screening "for changes in morphology or colony growth", but I could not find explanation of how the screen was performed in the methods. Please add this information.

p. 13: Pak1/Shk1 is not only a negative regulator of Cdc42. It is also one of the main effectors and is thought to play an important role in positive feedback.

p. 14: I do not understand well the argument around the use of orb2-34. The fact that the rga4Δ orb2-34 double mutant is monopolar is not particularly surprising, as orb2-34 is monopolar, and I could not see the link between this and the "polarisome".

p. 15: F. Chang is at UCSF

I hope you can address these points and those of the reviewers and will be happy to consider a revised manuscript.

Sincerely,

Sophie Martin
Monitoring Editor
Molecular Biology of the Cell

Dear Prof. Verde,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter above and the reviewer comments below.

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To submit the rebuttal letter, revised manuscript, and figures, use this link: Link Not Available

Please contact us with any questions at mboc@ascb.org.

Thank you for submitting your manuscript to Molecular Biology of the Cell. We look forward to receiving your revised paper.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

Fission yeast (S. pombe) is a model organism used to study symmetry breaking of polarity protein Cdc42. In wild-type S. pombe Cdc42 oscillates between two cell tips once the cell reaches a certain length threshold. Previous mathematical modeling shows that a switch occurs from asymmetric (monopolar) to symmetric (bipolar) distribution of Cdc42 as the cell grows. Bifurcation analysis predicts the existence of a co-existence of symmetric and asymmetric states in a certain parameter regime. This work focuses on the role of Cdc42 GAP rga4 (one of three GAPs in that system) in determining the distribution of Cdc42 in fission yeast cells. Following cell division, wild-type cells initially exhibit an asymmetric distribution of Cdc42, where most of Cdc42 is found at the old end (OE) and cells only elongate from that end. As cells grow in size, Cdc42 appears at the new end (NE) and bipolar growth begins in a process called NETO. In cells lacking rga4, this process is disrupted. Monopolar rga4null mothers always give rise to one monopolar and one bipolar cell. Daughters of bipolar mothers remains monopolar.
This work tries to characterize what gives rise to this inheritance pattern. The experimental findings are: (1) localization of Cdc42 scaffold Scd2 is not altered in rga4null cells. This means that Cdc42 can continue to bind to both tips. (2) Decreasing size of bipolar rga4null daughter cells abolishes bipolar growth. (3) Another regulator of Cdc42, GAP rga6, is asymmetrically inherited during cell division in monopolar rga4null cells, but not in bipolar ones. The GEFs do not display visible differences in inheritance. In the discussion, the authors conclude that "our results support the notion that divergent rga4null daughter cells are born with distinct initial conditions, resulting in different growth patterns"

This work is interesting, I find it well-written and the experimental results are well-presented. However, as a theoretician I found the presentation of three hypotheses that can potentially explain these results to be confusing. This is particularly problematic, as the model of Cdc42

oscillations which is modified to explain these results is a well-mixed model that does not include cell volume, even as a parameter. Thus makes presentation of hypothesis 2 that daughters of rga4null cells inherit unequal volumes particularly difficult to follow. Instead the authors modify positive feedback parameter Csat as a proxy for decreased GAP activity.

Here are more detailed comments:

1) in hypothesis 1, a new time-dependent aging factor is added in front of Cdc42 association constant lambda+, as well as modifying positive feedback parameter Csat and parameter epsilon (that appears in dissociation rate k-) from the original model published in Das (2012). The description of epsilon provided is that it is set to match oscillation size. I found Supplemental Figure 2 to be confusing as WT cells also have a time-dependent aging parameter, so it does not reduce to the Das model for WT cells. Are the authors proposed an alternative to the Das 2012 model then, or do they still think that the 2012 model captures wildtype behaviour?

2) Rga4 is a negative regulator of Cdc42. However, all model variants consider variations from WT values in both activation of Cdc42 (Csat resulting in change in lambda+) rather than just in dissociation constant k-? Is there a justification (either biological or mathematical) for this assumption?

3) The treatment of unequal volumes for daughter cells in hypothesis 2 should be made clearer in model description, as volume does not appear in the mathematical model. Maybe the authors can provide a short description how each parameter in the model scales with volume. The authors may be interested that in the stochastic version of the model by Xu and Jilkine, changing the volume of the cell, while keeping the total amount of Cdc42 and parameters fixed results in different behaviours with stochastic fluctuations leading to random switching from a stable limit cycle to a steady state and back, or extinguishing oscillations altogether. Also, coherence resonance is observed when stochasticity is included in that model, that is, quasi-cycles are observed in the stochastic model where the deterministic model is predicted to have damped oscillations.

Ref: Xu, Bin, Hye-Won Kang, and Alexandra Jilkine. "Comparison of deterministic and stochastic regime in a model for Cdc42 oscillations in fission yeast." Bulletin of mathematical biology 81.5 (2019): 1268-1302.

4) Hypothesis 3 is presented clearer than hypothesis 1 or 2. There, lambda+ is a time-dependent parameter compared to the basal model, and after unequal division lambda+ starts at different levels in daughters of monopolar mothers. However, the authors should be a bit careful. It is difficult to separate cause and effect here. Is Rga6 inherited asymmetrically and then establishes asymmetric Cdc42 distribution, or is it because it is bound to active Cdc42 that is inherited asymmetrically? Finally, all the model simulation findings should be discussed under Results not Discussion.

5) I know Supplemental Fig.1A is meant to recapitulate the already published Das model from 2012. However, it is a bit confusing, as the presented phase portraits only show the stable steady states, not the periodic trajectories, and at first glance do not make sense from a topological point of view, as any periodic trajectory must have enclose fixed points whose indices sum up to 1 (See Strogatz, Nonlinear Dynamics and Chaos, Chapter 6). Would it be possible to also indicate unstable fixed

points in a different color like black, and also indicate what shape the periodic trajectory would take in the phase plane when it exists?

Reviewer #2 (Remarks to the Author):

Review
In this paper Rodrigues-Pino et al study how the loss of the GAP rga4 effects the previously observed both bistability in monopolar and biopolar growth of daughter cells. They combine a genetics approach where they study different mutants and interpret them in the light of a previously published mathematical model and find that rga6 (another GAP) most strongly enhances the effects of rga4. I appreciate the approach to formulate hypothesis more formally using a mathematical model. However while reading the paper the model and experiments appear disconnected. The paper leans strongly on a paper published by the same group in 2012.
While reading the paper it remains unclear what kind of model is used, only that there is a model (this is a generic statement). So is it a molecular, course-grained, 1D/2D/3D for example? Only until you dive into the supplements it become clear. I do not think it is necessary to describe the model in detail in the main text but at least the key ingredients should be introduced. Especially because it is actually a rather clean and understandable model.
One major concern I have is that the model is more a hypothesis generator, rather than that it can be directly tested by the experiments. Given that more molecular models exist, I would like to know why the authors did not make a molecular model that is more close to the experiments they performed, and thus also more testable. If I read the main conclusion or the title, it is not clear to me the model was necessary to reach these conclusions.
Another concern I have is that one of the main findings is that rga6 has similar effects and enhances effects of rga4. The authors discuss this finding in the light of having different concentrations of regulators present (as this is one of their model hypothesis). However rga4 and rga6 are very similar regulators, they are both GAPs, and may have similar effects and just enhance each other through the same (unknown) mechanisms (because by deleting both the concentration of GAPs is even lower). I would like the to see that also reflected in the discussion, where now more generally the effects of regulators is discussed.
My last major concern is that overall I find the paper difficult to understand. Often terms are introduced where it is not clear to me what they mean. For example, is the state of Cdc42 activation, different from the distribution of active and inactive Cdc42 in the cell? Also the figures are at several occasions not intuitive/complete. As a consequence I find it hard to verbalize for myself what the main claims and findings of this paper are. Probably rewriting it, such that it becomes more independent from the 2012 paper will help.
I will give examples and minor comments below.
p.6 "different computational models" are these completely different models, or variations in the same model (I can check this in the supplement but I think it should be more clear here)
Suppl fig 1A: this is outcome is to some extend generic for such a set of equations. In this case what can vary is the lengths of the arrows (depending on the parameter choice), I guess it is not clear to me what I should take from this figure?
P7 does CRIB-GFP not perturb the dynamics of polarization? Did you, (or someone previously), check this with Antibody staining or with precise growth rate experiments?
Figure 1B: I do not understand the y-axis. And the x-axis, is this a histogram for multiple cells or one cell growing over time, or multiple (how many) cells combined growing over time?
Figure 1CDEF what is the unit of the y-axis and how is this determined and normalized (should at least briefly be described)?

p.8 "as the cell increases in size total levels of Cdc42 increases" Did people check in Pombe that Cdc42 concentration is constant over the cell cycle, thus that there is no regulation?
P13 "Conversely" Here I am confused. How I read it in both cases monopolar cells have their OE labelled and bipolar cell both ends.
Figure 5: I do not think one should assume that the reader knows why Rlc1 and Calcofluor are used and what they indicate. And I would add the statistics from the suppl to this figure (and also add statistics to the triple mutant)
Figure 6: why not add statistics to all the four cases in A? Same for figure 7c?

We thank the Editor and the Reviewers for the helpful comments. We have endeavored to address the comments and update the paper, by changing the text and adding experiments, and we feel it is much improved. Please see below our response.

Editor's comments:

1. None of the three hypotheses explored in the models are conclusively refuted or supported by the experiments. I would ask that, in addition to addressing the comments made by the reviewers, you consider the two following main points and more minor points below.

First, your main conclusion is that unequal inheritance of the GAP Rga6 underlies the asymmetric growth patterns of rga4Δ daughter cells. You show that the two daughters of rga4Δ monopolar cells have different Rga6 levels and that rga6Δ enhances the shape asymmetry between daughter cells, but what happens to the growth patterns in these cells? This would be easy and important to test. If differential Rga6 levels contribute to growth pattern asymmetry, deleting it should make the two daughter cells more equal in growth patterns.

A: To answer the Editor's question, we followed the growth pattern of 10 *rga4Δ rga6Δ* cell pairs (see Supplementary Figure 4B. We found that the overall pattern of growth is similar to the *rga4Δ* cells: monopolar cells give rise to daughter cells with divergent patterns of growth (Supplementary Figure 4B, a, b, c), and bipolar *rga4Δ rga6Δ* cells give rise to monopolar daughter cells (Supplementary Figure 4B, c and d). *rga4Δ rga6Δ* cells also exaggerate the divergence in cell size expansion already observed in *rga4Δ* daughter cells (Supplementary Figure 2, C).

These effects may be explained, in part, by the presence of the last remaining Cdc42 GAP, Rga3. Rga3 localization varies in *rga4Δ rga6Δ* cells (Supplementary Figure 6A), and appears more asymmetrically localized in dividing monopolar *rga4Δ rga6Δ* cells (Supplementary Figure 6A, a), although Rga3GFP quantification is very challenging due to the enlarged shape of cells and faintness of the signal. Consistent with a residual role for Rga3 in *rga4Δ rga6Δ* pattern of growth, removing Rga3 as well leads to circular cells with an almost complete loss of polarity after cell division (Supplementary Figure 6B), as previously reported in interphase cells (Gallo Castro and Martin, 2018). These additional data is mentioned in page 17.

Second, some of the experiments are difficult to interpret and these difficulties should be openly acknowledged in the text. In particular, the interpretation of effect of cell size is complicated by the fact that pom1Δ cells are themselves largely monopolar. It thus seems rather unsurprising that the double mutants are also monopolar, and difficult to specifically attribute this change to a change in cell size. The difficulty in interpretation is similar for the gef1Δ rga4Δ experiments, as cells lacking gef1Δ are themselves monopolar.

A: We added a discussion of the *pom1* deletion effect, providing alternative interpretations. We found it striking that *pom1* deletion decreases the size of the *rga4Δ* cell that is destined to become bipolar, correcting the pattern of growth. It is also an interesting fact that the predominance of the old end is mostly restored in these cells. However, it is indeed possible that loss of Pom1 may affect polarization in these cells by regulating other cellular functions, such as Mod5 and Tea4, and multiple GTPase signaling networks involved in cell polarity. We acknowledged this possibility in the text (page 15).

Conversely, it is more likely that deletion of *gef1* delays bipolar cell growth, as it is predicted in our model, because Cdc42 activity is overall decreased in *gef1Δ* cells. So we think that this result is not

trivial, but that it reflects the fact that in *rga4Δ* bipolar cells Cdc42 activity is higher. Consistent with this idea, we found that bipolar *rga4Δ* cells grow faster that monopolar *rga4Δ* cells. We added this data to the paper (Supplementary Figure 2, C) and added a mention in the text (page 9).

Other clarification needed:

It would be nice to provide supplementary movies to Fig 2A to show the interesting pedigree growth pattern of rga4Δ cells.
A: We added one control movie, two movies of the *rga4Δ* phenotype, and a Supplementary figure showing the quantification of cell growth in control and *rga4Δ* cells (Supplementary Figure 2).

In Fig 5B, the rga4Δ cell marked as bipolar does not look very bipolar compared to the monopolar one shown above: the distance from the birth scar to the cell end seems quite similar in the two cells.
A: We changed Figure 5g, adding a clearer example.

I have trouble understanding the quantification of the pom1Δ rga4Δ experiment in Fig 6. The text states that all monopolar cells gave rise to two monopolar daughters (100%), but the panels B and C show some bipolar cells. What is the frequency of the bipolar pattern?
A: For the graphs in Fig.6, B and C, we analyzed 19 monopolar and 5 bipolar *pom1Δ rga4Δ* cells (see Figure 6B legend). Interestingly, these numbers are in line with the previously reported analysis by the Dai Hirata lab (Koyano et al., 2010), which found a roughly 3:1 ratio of monopolar to bipolar cells in *pom1Δ* cells. The number of cells that were followed under the microscope in time-lapse analysis was actually 15 (not 10, we corrected in the text). However, not all of these cells were followed all the way to the next division, so we may have missed the small number that went on to activate NETO. We addressed this point in the text (page 15).

p. 10: It would be helpful to the reader if you provide a more extensive explanation of the parameters that are changed in the model.
A: We addressed this in the text on page 10, adding additional explanation and discussion and modifying it in response to comments by Reviewer 1. The explanation of parameter λ^{+}_{0} is now provided within hypothesis 3.

p. 12: You explain you performed an SGA screen, by visual screening "for changes in morphology or colony growth", but I could not find explanation of how the screen was performed in the methods. Please add this information.
A: We added an explanation in the Material and Methods to describe how we performed the SGA screen.

p. 13: Pak1/Shk1 is not only a negative regulator of Cdc42. It is also one of the main effectors and is thought to play an important role in positive feedback.
A: Indeed these are important points. We were not clear in our explanations, including the fact that the *orb2-34* allele has a specific mutation disrupting kinase activity. We have added a mention of the different functions of Pak1 in the Introduction on page 5.

p. 14: I do not understand well the argument around the use of orb2-34. The fact that the rga4Δ orb2-34

double mutant is monopolar is not particularly surprising, as orb2-34 is monopolar, and I could not see the link between this and the "polarisome".
A: We agree that this experiment is not particularly helpful. For the sake of clarity, we decided to eliminate the *orb2-34* findings from the paper, and keep only the results that came from the SGA screen using the Bioneer library.

p. 15: F. Chang is at UCSF
A: We changed the text with the wrong affiliation.

Reviewer #1 (Remarks to the Author):

Fission yeast (S. pombe) is a model organism used to study symmetry breaking of polarity protein Cdc42. In wild-type S. pombe Cdc42 oscillates between two cell tips once the cell reaches a certain length threshold. Previous mathematical modeling shows that a switch occurs from asymmetric (monopolar) to symmetric (bipolar) distribution of Cdc42 as the cell grows. Bifurcation analysis predicts the existence of a co-existence of symmetric and asymmetric states in a certain parameter regime. This work focuses on the role of Cdc42 GAP rga4 (one of three GAPs in that system) in determining the distribution of Cdc42 in fission yeast cells. Following cell division, wild-type cells initially exhibit an asymmetric distribution of Cdc42, where most of Cdc42 is found at the old end (OE) and cells only elongate from that end. As cells grow in size, Cdc42 appears at the new end (NE) and bipolar growth begins in a process called NETO. In cells lacking rga4, this process is disrupted. Monopolar rga4null mothers always give rise to one monopolar and one bipolar cell. Daughters of bipolar mothers remains monopolar.
This work tries to characterize what gives rise to this inheritance pattern. The experimental findings are: (1) localization of Cdc42 scaffold Scd2 is not altered in rga4null cells. This means that Cdc42 can continue to bind to both tips. (2) Decreasing size of bipolar rga4null daughter cells abolishes bipolar growth. (3) Another regulator of Cdc42, GAP rga6, is asymmetrically inherited during cell division in monopolar rga4null cells, but not in bipolar ones. The GEFs do not display visible differences in inheritance. In the discussion, the authors conclude that "our results support the notion that divergent rga4null daughter cells are born with distinct initial conditions, resulting in different growth patterns"

This work is interesting, I find it well-written and the experimental results are well-presented. However, as a theoretician I found the presentation of three hypotheses that can potentially explain these results to be confusing. This is particularly problematic, as the model of Cdc42 oscillations which is modified to explain these results is a well-mixed model that does not include cell volume, even as a parameter. Thus makes presentation of hypothesis 2 that daughters of rga4null cells inherit unequal volumes particularly difficult to follow. Instead the authors modify positive feedback parameter Csat as a proxy for decreased GAP activity.

A: This point was not clearly explained. The model does include cell volume by assuming that volume and total amount of limiting component (C_{tot}) increases with cell length. We have now clarified this by adding a sentence in hypothesis (2) on page 11 and below Eq. (1) of Supplemental text. We also explain the limitations of the assumption of a well-mixed model below Eq. (2) in Supplemental text. A recent model with Cdc42 diffusion along the membrane (bulk diffusion should be sufficiently fast over the time scale of oscillations) showed that may Cdc42-GDP membrane diffusion may play a role, however it was not a limiting factor (Khalili et al. Cells 2020). For this reason, we still believe the current model is the

simpler one to consider first, since it includes in the simplest form the basic mechanisms of competition, saturation, positive and negative feedbacks .

Here are more detailed comments:

1) in hypothesis 1, a new time-dependent aging factor is added in front of Cdc42 association constant lambda+, as well as modifying positive feedback parameter Csat and parameter epsilon (that appears in dissociation rate k-) from the original model published in Das (2012). The description of epsilon provided is that it is set to match oscillation size. I found Supplemental Figure 2 to be confusing as WT cells also have a time-dependent aging parameter, so it does not reduce to the Das model for WT cells. Are the authors proposed an alternative to the Das 2012 model then, or do they still think that the 2012 model captures wildtype behaviour?
A: Also this point was not clearly stated. Since aging is additional mechanism to the model that should exist for both wild type and *rga4*Δ cells, the model with aging is indeed an alternative model to the Das 2012. However, we argue that the aging model should still provide the Das 2012 dynamics when applied to the wild type case (where both tips get a chance to age such that aging in effect drops out, approximately). We have updated the description of Hypothesis 1 to clarify this point.

2) Rga4 is a negative regulator of Cdc42. However, all model variants consider variations from WT values in both activation of Cdc42 (Csat resulting in change in lambda+) rather than just in dissociation constant k-? Is there a justification (either biological or mathematical) for this assumption?
A: One of the limitations with working with an effective model is that its parameters combine several molecular processes, included GAP-mediated inactivation. Parameter C_{sat} that sets the saturation threshold (resulting from a balance of activation and inactivation) would include GAP-mediated inactivation. This has been now stated explicitly. We have also clarified that Parameter ε describes the strength of the delayed negative feedback so this is also consistent with the effects of a negative regulator. We agree that it's less clear how the linear activation rate constant λ^{+}_{0} could represent a balance between activation and inactivation, so we moved the discussion on λ^{+}_{0} within hypothesis 3, where the regulators inherited asymmetrically could be positive or negative.

3) The treatment of unequal volumes for daughter cells in hypothesis 2 should be made clearer in model description, as volume does not appear in the mathematical model. Maybe the authors can provide a short description how each parameter in the model scales with volume. The authors may be interested that in the stochastic version of the model by Xu and Jilkine, changing the volume of the cell, while keeping the total amount of Cdc42 and parameters fixed results in different behaviours with stochastic fluctuations leading to random switching from a stable limit cycle to a steady state and back, or extinguishing oscillations altogether. Also, coherence resonance is observed when stochasticity is included in that model, that is, quasi-cycles are observed in the stochastic model where the deterministic model is predicted to have damped oscillations.

Ref: Xu, Bin, Hye-Won Kang, and Alexandra Jilkine. "Comparison of deterministic and stochastic regime in a model for Cdc42 oscillations in fission yeast." Bulletin of mathematical biology 81.5 (2019): 1268-1302.
A: We have now clarified in the description of hypothesis 2 as well as below Equation (1) in the supplement that changes of volume correspond to changes in parameter C_{tot}. We have also added a reference to the reference suggested by the reviewer when discussing prior modeling works as well as in the context of the effects of noise near the end of the Discussion section.

4) Hypothesis 3 is presented clearer than hypothesis 1 or 2. There, lambda+ is a time-dependent parameter compared to the basal model, and after unequal division lambda+ starts at different levels in daughters of monopolar mothers. However, the authors should be a bit careful. It is difficult to separate cause and effect here. Is Rga6 inherited asymmetrically and then establishes asymmetric Cdc42 distribution, or is it because it is bound to active Cdc42 that is inherited asymmetrically? Finally, all the model simulation findings should be discussed under Results not Discussion.
A: While we don't specify the mechanism by which regulators of Cdc42 might be inherited unequally, we do assume that such an unequal inheritance results from a monopolar growth pattern (unequal inheritance does not necessarily require binding to Cdc42 itself, it could also be due to long-lived protein bound states near or around the Cdc42 growth zone). Such a mechanism could lead to a self-sustained growth pattern similar to that of *rga4*Δ cells. We have added wording to clarify this when describing hypothesis 3.

We agree with the reviewer that it's preferable to have all results presented before the Discussion section. However, given the complexity of the arguments and the speculative aspects of Supplemental Fig. 7, we left the associated text in the Discussion section.

5) I know Supplemental Fig.1A is meant to recapitulate the already published Das model from 2012. However, it is a bit confusing, as the presented phase portraits only show the stable steady states, not the periodic trajectories, and at first glance do not make sense from a topological point of view, as any periodic trajectory must have enclose fixed points whose indices sum up to 1 (See Strogatz, Nonlinear Dynamics and Chaos, Chapter 6). Would it be possible to also indicate unstable fixed points in a different color like black, and also indicate what shape the periodic trajectory would take in the phase plane when it exists?
A: Thank you for this suggestion. We have now added the unstable fixed point in black and specified that these phase portraits correspond to the model without delayed negative feedback where there are no oscillations (in the absence of noise). We have also added a separate row of panels to indicate the periodic trajectories in the presence of delayed negative feedback. We note that since the flow pattern in the model with delayed negative feedback depends on past history, we cannot directly apply the index theorem for systems where flow is uniquely determined by position on phase space.

Reviewer #2 (Remarks to the Author):

Review
In this paper Rodrigues-Pino et al study how the loss of the GAP rga4 effects the previously observed both bistability in monopolar and biopolar growth of daughter cells. They combine a genetics approach where they study different mutants and interpret them in the light of a previously published mathematical model and find that rga6 (another GAP) most strongly enhances the effects of rga4. I appreciate the approach to formulate hypothesis more formally using a mathematical model. However while reading the paper the model and experiments appear disconnected. The paper leans strongly on a paper published by the same group in 2012.

While reading the paper it remains unclear what kind of model is used, only that there is a model (this is a generic statement). So is it a molecular, course-grained, 1D/2D/3D for example? Only until you dive into the supplements it become clear. I do not think it is necessary to describe the model in detail in the

main text but at least the key ingredients should be introduced. Especially because it is actually a rather clean and understandable model.
A: Thank you for this suggestion. We have now clarified that it is delayed differential equation model with 3 populations (two tips and cytoplasm) and mass conservation on page 4 as well as at the beginning of the Results section.

One major concern I have is that the model is more a hypothesis generator, rather than that it can be directly tested by the experiments. Given that more molecular models exist, I would like to know why the authors did not make a molecular model that is more close to the experiments they performed, and thus also more testable. If I read the main conclusion or the title, it is not clear to me the model was necessary to reach these conclusions.
A: Unfortunately, we do not yet know enough about the molecular mechanisms to generate reliable predictions of the large scale cell growth pattern starting from a microscopic model. A tentative, more detailed model, with explicit GEF and GAP concentrations has been proposed by some of us recently (Khalili et al, Cells 2020), however this model requires further validation to become predictive. Here we focused on a more coarse-grained model that still includes the main established mechanisms: tip activation, competition for a limiting component, saturation, positive feedback, and delayed negative feedback. These mechanisms, when formulated as a mathematical model, describe the growth pattern of wild type cells, while *rga4*Δ cells exhibit a different growth pattern. The question of what type of modification of the model is needed to obtain the *rga4*Δ pattern is a mathematical question that we believe is close to our experiments and whose answer is part of our results. We thus propose three hypotheses that we tried to test experimentally and which we found to be useful in interpreting our experiments.

Another concern I have is that one of the main findings is that rga6 has similar effects and enhances effects of rga4. The authors discuss this finding in the light of having different concentrations of regulators present (as this is one of their model hypothesis). However rga4 and rga6 are very similar regulators, they are both GAPs, and may have similar effects and just enhance each other through the same (unknown) mechanisms (because by deleting both the concentration of GAPs is even lower). I would like the to see that also reflected in the discussion, where now more generally the effects of regulators is discussed.
A: We added this important comment in the Discussion on Page 24

My last major concern is that overall I find the paper difficult to understand. Often terms are introduced where it is not clear to me what they mean. For example, is the state of Cdc42 activation, different from the distribution of active and inactive Cdc42 in the cell? Also the figures are at several occasions not intuitive/complete. As a consequence I find it hard to verbalize for myself what the main claims and findings of this paper are. Probably rewriting it, such that it becomes more independent from the 2012 paper will help.
A: We have gone through the paper and figures to try and sharpen some of our language and clarified/reworded occurrences of “state of Cdc42 activation” to clarify this means a state of the distribution of active and inactive Cdc42 in the cell (or, equivalently, a state of Cdc42 activation distribution).

I will give examples and minor comments below.
p.6 "different computational models" are these completely different models, or variations in the same model (I can check this in the supplement but I think it should be more clear here)
A: We have rephrased that sentence as follows "we tested the predictions of variations of our computational model for wild type cells, which suggested three alternative hypotheses to reproduce the unequal fate of *rga4Δ* daughter cells".

Suppl fig 1A: this is outcome is to some extend generic for such a set of equations. In this case what can vary is the lengths of the arrows (depending on the parameter choice), I guess it is not clear to me what I should take from this figure?
A: In this figure we want to summarize the results of the model of Das et al. in a graphical manner, to show the possible number of symmetric and asymmetric states. Also to indicate that changing parameters leads not only to change in the length of the arrows but also to changes between different number of fixed points (and thus, states of Cdc42 activation distribution). We have added this statement to the figure caption. We hope this figure is now more informative, also after the update in response to comments by Reviewer 1.

P7 does CRIB-GFP not perturb the dynamics of polarization? Did you, (or someone previously), check this with Antibody staining or with precise growth rate experiments?
A: CRIB-GFP does not perturb the dynamics of cell polarization (Tatebe aet al, 2008; Das et al., 2012) or the distribution of Cdc42 enrichment at cell poles (Bendezu' et al., 2015).

Figure 1B: I do not understand the y-axis. And the x-axis, is this a histogram for multiple cells or one cell growing over time, or multiple (how many) cells combined growing over time?
A: This is a distribution of CRIB-GFP at the two ends of a population of cells. The Y axis shows the relative distribution of CRIB-GFP at the two tips for each cell (where 0.5 value means that approximately same amounts of CRIB-GFP are present at both tips). The X axis places cells in different bins according to their length (the longer the cells is, the more likely it is that similar amounts of CRIB-GFP are present at both tips in the population). We quantified 61 wild-type, 45 *rga4Δ* bipolar and 61 *rga4Δ* monopolar cells. The heatmaps were generated using the R software (http://www.r-project.org/) as previously done (Das, M. *et al.* 2015). We added explanatory text to the Material and Methods.

Figure 1CDEF what is the unit of the y-axis and how is this determined and normalized (should at least briefly be described)?
A: We clarified in the Material and Methods Section text. The Units are arbitrary, and the quantification was performed as described in Das et al., 2012 and Das et a., 2015. We have added the A.U. description to Figure 1.

p.8 "as the cell increases in size total levels of Cdc42 increases" Did people check in Pombe that Cdc42 concentration is constant over the cell cycle, thus that there is no regulation?
A: Cdc42 protein concentrations do not vary during the cell cycle as determined by absolute proteomic dynamics (Carpy et al., 2014). In the text we mention total levels of "active Cdc42".

P13 "Conversely" Here I am confused. How I read it in both cases monopolar cells have their OE labelled and bipolar cell both ends.
A: We have eliminated "conversely", since it was confusing.

Figure 5: I do not think one should assume that the reader knows why RIc1 and Calcofluor are used and what they indicate. And I would add the statistics from the suppl to this figure (and also add statistics to the triple mutant)

A: We added the explanation that Rlc1 is used to determine progression through cell division, and calcofluor to determine if a cell is growing in a monopolar or bipolar fashion. We changed figure 5 to include the statistics originally in the Supplemental Section.

Figure 6: why not add statistics to all the four cases in A? Same for figure 7c?

A: While we have the control data in the case of Figure 6A, we decided not to add the statistics for the *pom1Δ* cells. We found, as previously reported by other groups, that many *pom1Δ* cells display a "slanted" septum at the time of division. This makes the quantification of length and volume of the two cell compartments at division very difficult. This phenotype was not prevalent with the *rga4Δ pom1Δ* cells, so we decided to show the comparison of *rga4Δ* and *rga4Δ pom1Δ* only.

In the case of Figure 7c, we wanted to avoid confusion by adding other possible growth patterns. WT cells activate bipolar growth (NETO) later in the cell cycle. Also *gef1Δ* cells, albeit mostly monopolar, become bipolar to a smaller percentage. We are indicating in the figure that loss of *gef1* alters the *rga4Δ* growth pattern, in the described fashion.

RE: Manuscript #E20-10-0666R
TITLE: "Cdc42 GTPase Activating Proteins (GAPs) Regulate Generational Inheritance of Cell Polarity and Cell Shape in Fission Yeast"

Dear Fulvia,

Thank you for sending your revised manuscript to MBoC and apologies for the delay in my response, due to the late arrival of one of the reviews. As you will see from the reviewers, these find your manuscript improved, but still have some comments, which I believe can be addressed by text changes. I leave it up to you to change the order of presentation of the models or not, as suggested in point 1 of reviewer 1.

I would be grateful if you can submit with your revision a track-change version of the manuscript, which would help me scan through the changes you have made. I should then be able to make a decision without further consultation with reviewers.

Best wishes,
Sophie

Monitoring Editor
Molecular Biology of the Cell

Dear Prof. Verde,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments (if any) below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

Authors are allowed 180 days to submit a revision. If this time period is inadequate, please contact us immediately at mboc@ascb.org.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your

revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised version, and figures, please use this link (please enable cookies, or cut and paste URL): Link Not Available

Authors of Articles and Brief Communications whose manuscripts have returned for minor revision ("revise only") are encouraged to create a short video abstract to accompany their article when it is published. These video abstracts, known as Science Sketches, are up to 2 minutes long and will be published on YouTube and then embedded in the article abstract. Science Sketch Editors on the MBoC Editorial Board will provide guidance as you prepare your video. Information about how to prepare and submit a video abstract is available at www.molbiolcell.org/science-sketches. Please contact mboc@ascb.org if you are interested in creating a Science Sketch.

Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

While the authors provide some explanations in their rebuttal letter, and have clarified the verbal description of the model in the main text of the paper, very little changes have actually been done to the Section on Mathematical Model in Materials & Methods. Supplemental Material provides an updated supplementary figure 1, as requested, but has no additional information on the models. Since, the readers will not have access to the rebuttal letter, I ask the authors to clarify some outstanding issues I brought up in my original review, which I state again, below. Note that I am not trying the authors to change their model, but just to make sure all the assumptions have been clearly laid out for the interested reader, and put in enough detail, so that others can replicate their results without having to guess about what the authors meant.

Again, while I find the described experimental results interesting, and the description of the three model variants in the main text has been significantly improved, I still find a disconnect between the paper narrative about the effect of GAPs on inheritance on cell polarity and what is actually being changed in each variant of the model in this version of the manuscript.
Finally, can the revised version have the changes that have been made highlighted for the ease of reviewing?

Here are more detailed comments:

1. The authors state in rebuttal letter that "Since aging is additional mechanism to the model that should exist for both wild type and rga4Δ cells, the model with aging is indeed an alternative model to the Das 2012. However, we argue that the aging model should still provide the Das 2012

dynamics when applied to the wild type case (where both tips get a chance to age such that aging in effect drops out, approximately). We have updated the description of Hypothesis 1 to clarify this point."

My comment: Would it not make more sense then to present hypothesis 2 first, as it uses the same equations as the original Das model, which is presented in equations 1-3, while hypothesis 1 (equations 4) and 3 (equation 5) modifies that model? This presentation would also make more sense, since both hypotheses 1 and 3 effectively modify the parameter lambda^+_0, while hypothesis 2 does not. The authors also state in the rebuttal letter that "We agree that it's less clear how the linear activation rate constant λ+ 0 could represent a balance between activation and inactivation, so we moved the discussion on λ+ 0 within hypothesis 3, where the regulators inherited asymmetrically could be positive or negative," suggesting that this re-organization may be easier.

2. The description of hypothesis 2 is limited to "Daughters of monopolar rga4Δ mothers inherit unequal volumes. The equations for this model are the same as the ones in (Das et al., 2012). We study the behavior of cells that start with different total amounts Ctot, in proportion to the differences in volume." However, it is not stated either in the text or figure 3 legend, what are the Ctot values used for different daughter cells. Also,does Ctot have units (those aren't indicated in the Table), and how were the values for Ctot chosen? Were they based on any sort of empirical measurement?
3. My original comment on treatment of cell volume in the equations was not fully addressed. The authors only inserted a sentence that "The total amount is tot = Ccyt + 1 + C2 and is assumed to increase in proportion to cell volume with constant rate tot/." However, if cell volume is included, the correct mass conservation statement is
AC1+AC2+V Ccyt=VCtot, where A is the cross-sectional area, which reduces to replacing Ccyto in eq (1-3) by Ccyt=Ctot-A/V(C1+C2). So both Ccyt and Ctot should be modified as the cell volume changes. Is this what the authors actually did or did they do something else? And then how did they modify this to account for different volumes of the daughter cells, and what exactly, was changed between between Fig 3A (wild-type cells) and Fig 3C (hypothesis 2)? Note that the figure output shows C1/Ctot and C2/Ctot, so it is unclear to the reader how Ctot got changed for the 2 daughter cells.
4. Please provide a complete mathematical description of the model used to produce the figure 3 with ICs used for the simulation rather than just a verbal description. Also, since a DDE rather than a DE is used, what is the initial history function and how does it get adjusted for hypothesis 1 (tip aging)? On a related minor note why does the y axis state "c1 over Ctot". I presume the readership of Molecular biology of the Cell understands fractions.
5. Again, I mentioned previously to the authors that simply changing the volume of the cell, while keeping the total amount of Cdc42 fixed (which is what is done in hypothesis 2!) in a stochastic model can result in different behaviours, with stochastic fluctuations leading to random switching from a stable limit cycle to a steady state and back, or extinguishing oscillations altogether. This means that stochastic implementations of their 3 variants may give rise to different results than the deterministic results presented here. I don't see that addressed anywhere in discussion of results.
Ref: Xu, Bin, Hye-Won Kang, and Alexandra Jilkine. "Comparison of deterministic and stochastic regime in a model for Cdc42 oscillations in fission yeast." Bulletin of mathematical biology 81.5 (2019): 1268-1302.
6. The opening sentence "Cell polarization is an important process that enables essential cellular functions such as morphogenesis, cell migration, and asymmetric cell division" cites Turing's 1952 paper on the chemical basis of morphogenesis. While Turing's 1952 work is a seminal paper in mathematical biology, it states nothing on the subject of cell polarization or has anything to do with

the models being presented (since those models do not include diffusivity of the proteins). On P. 10- should say "delay differential equation" rather than differential equation.

Reviewer #2 (Remarks to the Author):

The authors have answered several of my comments and have improved the manuscript. The figures are now easier to interpret, although putting a brief explanations in the captions, rather than only in the supplements may still help.

Overall the data is convincingly showing a role for Rga6. So I would suggest that the authors focus their abstract more on the experimental results, because the connection between the model and experiments remains weak. In the introduction more molecular models are discussed now, however there is no conclusion based on this review of molecular models which justifies their phenemenological model choice for this specific problem. Especially because of the claim in the intro that they will "..identify novel molecular mechanisms that control morphological differentiation" which is not justified by the phenomenological model.

So overall the paper would still benefit from a more clear and sharp research question and story line: What are they after, are they after molecular mechanisms? Or a more phenomenological description, which is also fine, but now the paper does not chose and tries to unconvincingly do both.

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While the authors provide some explanations in their rebuttal letter, and have clarified the verbal description of the model in the main text of the paper, very little changes have actually been done to the Section on Mathematical Model in Materials & Methods. Supplemental Material provides an updated supplementary figure 1, as requested, but has no additional information on the models. Since, the readers will not have access to the rebuttal letter, I ask the authors to clarify some outstanding issues I brought up in my original review, which I state again, below. Note that I am not trying the authors to change their model, but just to make sure all the assumptions have been clearly laid out for the interested reader, and put in enough detail, so that others can replicate their results without having to guess about what the authors meant.

A. We are sorry for the lack of clarity. We have added additional information on the model assumptions as described in the detailed comments below.

Again, while I find the described experimental results interesting, and the description of the three model variants in the main text has been significantly improved, I still find a disconnect between the paper narrative about the effect of GAPs on inheritance on cell polarity and what is actually being changed in each variant of the model in this version of the manuscript.
Finally, can the revised version have the changes that have been made highlighted for the ease of reviewing?

A. We have reworded sentences in the manuscript to better clarify how the model inspired some of the experimental approaches, and vice versa. We are dealing with a complex system, hence the model is limited by our lack of detailed mechanistic understanding while experiments have their own practical limitations. However, we believe that progress in the field relies on building such cross-talk between theory and experiment to whatever extent is meaningful and possible. Thus, as long as our reasoning is not erroneous, we think that our work advances the field, even if the model and experimental parameters cannot be perfectly matched at the moment. The new changes to better explain our approach have been highlighted.

Here are more detailed comments:

1. The authors state in rebuttal letter that "Since aging is additional mechanism to the model that should exist for both wild type and rga4Δ cells, the model with aging is indeed an alternative model to the Das 2012. However, we argue that the aging model should still provide the Das 2012 dynamics when applied to the wild type case (where both tips get a chance to age such that aging in effect drops out, approximately). We have updated the description of Hypothesis 1 to clarify this point."

My comment: Would it not make more sense then to present hypothesis 2 first, as it uses the same equations as the original Das model, which is presented in equations 1-3, while hypothesis 1 (equations 4) and 3 (equation 5) modifies that model? This presentation would also make

more sense, since both hypotheses 1 and 3 effectively modify the parameter lambda^+_0, while hypothesis 2 does not. The authors also state in the rebuttal letter that "We agree that it's less clear how the linear activation rate constant λ+ 0 could represent a balance between activation and inactivation, so we moved the discussion on λ+ 0 within hypothesis 3, where the regulators inherited asymmetrically could be positive or negative," suggesting that this re-organization may be easier.

A. We agree with the reviewer's suggestion that placing hypothesis 2 first would make more sense mathematically. However, we would prefer to maintain the original order of presentation because it addresses the mechanisms that would be more likely biologically. Marking a growth site is a generally considered a more likely mechanism, so we decided to address it first.

2. The description of hypothesis 2 is limited to "Daughters of monopolar rga4Δ mothers inherit unequal volumes. The equations for this model are the same as the ones in (Das et al., 2012). We study the behavior of cells that start with different total amounts Ctot, in proportion to the differences in volume." However, it is not stated either in the text or figure 3 legend, what are the Ctot values used for different daughter cells. Also,does Ctot have units (those aren't indicated in the Table), and how were the values for Ctot chosen? Were they based on any sort of empirical measurement?

A. We had stated in section "Model Parameter Values" that "In all cases, amount at tips and cell middle measured with respect to the saturation parameter in the model of wild type cells without tip aging, C_{sat}^{WT}." So we work in units in which our reference total amount is $C_{sat}^{WT} = 1$. The two other parameters that inherit the units of C_{sat}^{WT}, λ_0^+ and λ_4^+, as well as the initial value of C_{tot} were selected to reproduce the asymmetric to symmetric transition during cell growth, as indicated in the Table of the same section. We now clarify that the indicated value of C_{tot}/C_{sat}^{WT} in the table is the initial value at birth. We also added to the table the final value of C_{tot}/C_{sat}^{WT} at division, which is twice the initial value, to the table. The values of C_{sat} for the mutant cells are indicated in the table.

For the model with cell aging, we had stated in the Model description that "Each daughter is assumed to start with half of the volume of the mother." We added the word "at division" for clarity.

For the model with unequal volumes, we had stated "… start with different total amounts C_{tot}, in proportion to the differences in volume". We added "compared to the initial value of wild type cells " for clarity.

We also added statements to clarify that the equations were integrated for a time corresponding to four hours.

3. My original comment on treatment of cell volume in the equations was not fully addressed. The authors only inserted a sentence that "The total amount is Ctot = Ccyt + C1 + C2 and is assumed to increase in proportion to cell volume with constant rate dCtot/dt." However, if cell

volume is included, the correct mass conservation statement is AC1+AC2+V Ccyt=VCtot, where A is the cross-sectional area, which reduces to replacing Ccyto in eq (1-3) by Ccyt=Ctot-A/V(C1+C2). So both Ccyt and Ctot should be modified as the cell volume changes. Is this what the authors actually did or did they do something else? And then how did they modify this to account for different volumes of the daughter cells, and what exactly, was changed between between Fig 3A (wild-type cells) and Fig 3C (hypothesis 2)? Note that the figure output shows C1/Ctot and C2/Ctot, so it is unclear to the reader how Ctot got changed for the 2 daughter cells.

A. We use symbol C (for Cdc42) to indicate total number (i.e. amount, not concentration). For cells that start with different volumes, we state that "cells start with different total amounts Ctot in proportion to the differences in volume". We added the initial values of Ctot in the legend of Figure 3 and added a statement "Thus, the three cases of Hypothesis 2 in Figure 3 correspond to different initial conditions for Ctot" in the description of this hypothesis.

4. Please provide a complete mathematical description of the model used to produce the figure 3 with ICs used for the simulation rather than just a verbal description. Also, since a DDE rather than a DE is used, what is the initial history function and how does it get adjusted for hypothesis 1 (tip aging)? On a related minor note why does the y axis state "c1 over Ctot". I presume the readership of Molecular biology of the Cell understands fractions.

A. We updated the table of model parameters to indicate the initial value of Ctot. We also now state how we initialize each tip and the history function. Since the system executes many oscillations within each asymmetric or symmetric state, and since we do not typically start within a coexistence region, the results are not sensitive to the precise choice of these initial values. We changed the labeling of the y-axis as the reviewer is suggesting.

5. Again, I mentioned previously to the authors that simply changing the volume of the cell, while keeping the total amount of Cdc42 fixed (which is what is done in hypothesis 2!) in a stochastic model can result in different behaviours, with stochastic fluctuations leading to random switching from a stable limit cycle to a steady state and back, or extinguishing oscillations altogether. This means that stochastic implementations of their 3 variants may give rise to different results than the deterministic results presented here. I don't see that addressed anywhere in discussion of results. Ref: Xu, Bin, Hye-Won Kang, and Alexandra Jilkine. "Comparison of deterministic and stochastic regime in a model for Cdc42 oscillations in fission yeast." Bulletin of mathematical biology 81.5 (2019): 1268-1302.

A. In hypothesis 2, which we hope is now better explained, we assume the total amount of Cdc42 is reduced in proportion to the reduction in cell volume. We agree that stochastic effects should be further investigated. A more involved hypothesis 4, which partly attributes the rga4 growth pattern to stochastic fluctuations is possible, however we reasoned that it's best to leave this possibility for future work due to the additional complexity of such a model, together with the very different required line of experimental investigation. We do now acknowledge

this phenomenon in the discussion, as well as acknowledge that we neglect noise when introducing the three hypotheses.

6. The opening sentence "Cell polarization is an important process that enables essential cellular functions such as morphogenesis, cell migration, and asymmetric cell division" cites Turing's 1952 paper on the chemical basis of morphogenesis. While Turing's 1952 work is a seminal paper in mathematical biology, it states nothing on the subject of cell polarization or has anything to do with the models being presented (since those models do not include diffusivity of the proteins). On P. 10- should say "delay differential equation" rather than differential equation.

A. We removed the reference to Turing and added the word “delay” on that page.

Reviewer #2 (Remarks to the Author):

The authors have answered several of my comments and have improved the manuscript. The figures are now easier to interpret, although putting a brief explanations in the captions, rather than only in the supplements may still help.

A. We added a brief explanation in the caption of Fig.3.

Overall the data is convincingly showing a role for Rga6. So I would suggest that the authors focus their abstract more on the experimental results, because the connection between the model and experiments remains weak. In the introduction more molecular models are discussed now, however there is no conclusion based on this review of molecular models which justifies their phenemenological model choice for this specific problem. Especially because of the claim in the intro that they will "..identify novel molecular mechanisms that control morphological differentiation" which is not justified by the phenomenological model.

So overall the paper would still benefit from a more clear and sharp research question and story line: What are they after, are they after molecular mechanisms? Or a more phenomenological description, which is also fine, but now the paper does not chose and tries to unconvincingly do both.

A. Following the reviewer’s advice we have rephrased the abstract, introduction and story line such that the model is indeed presented as a motivating, yet necessary phenomenological hypothesis. We also try to justify how, even though the models cannot yet be expected to uniquely match and predict precise molecular mechanisms, we do intend to "..identify novel molecular mechanisms that control morphological differentiation" and this process naturally goes through constructing mathematical models, even if phenomenological. For example, in the case of rga4 the pattern, mathematical models are needed to demonstrate the minimum number of assumptions to explain the rga4 pattern (qualitative descriptions are not sufficient

to show the mutual dependencies needed in a potentially very complex system). These are the three hypotheses, which is part of our results, used to interpret our experiments. In our added discussion we better justify the choice of the phenomenological hypotheses, which are the simplest while most general possibilities given the state of knowledge in the field.

RE: Manuscript #E20-10-0666RR
TITLE: "Cdc42 GTPase Activating Proteins (GAPs) Regulate Generational Inheritance of Cell Polarity and Cell Shape in Fission Yeast"

Dear Fulvia,

I thank you for your revisions and explanations, which have clarified all remaining questions, also for the reviewers. I am thus very happy to accept your manuscript "Cdc42 GTPase Activating Proteins (GAPs) Regulate Generational Inheritance of Cell Polarity and Cell Shape in Fission Yeast" for publication in Molecular Biology of the Cell. This study will provide an interesting theoretical and experimental exploration in the mechanisms of daughter cell symmetry.

Best wishes,
Sophie

Sophie Martin
Monitoring Editor
Molecular Biology of the Cell

Dear Prof. Verde:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

Within approximately four weeks you will receive a PDF page proof of your article.

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We are pleased that you chose to publish your work in MBoC.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

The authors have clarified the answers to my questions.